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Nanotechnology: Emerging Tool for Diagnostics and Therapeutics

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Abstract Nanotechnology is an emerging technology which is an amalgamation of different aspects of science and technology that includes disciplines such as electrical engineering, mechanical engineering, biology, physics, chemistry, and material science. It has potential in the fields of information and communication technology, biotechnology, and medicinal technology. It involves manipulating the dimensions of nanoparticles at an atomic scale to make use of its physical and chemical properties. All these properties are responsible for the wide application of nanoparticles in the field of human health care. Promising new technologies based on nanotechnology are being utilized to improve diverse aspects of medical treatments like diagnostics, imaging, and gene and drug delivery. This review summarizes the most promising nanomaterials and their application in human health.

Keywords Nanotechnology · Drug delivery · Gene delivery · Imaging · Diagnostics

Introduction

Nanotechnology is defined as the design and production of devices with special and improved properties which is due to their nanoscale preparations. Nanoscale is the length scale of approximately 1–100 nm range at which the particles are small enough to confine their electrons and produce quantum effects that results in novel properties. The properties of larger bulk particles are different from those acquired by particles of ultra small dimensions in nanometer range. The change in the properties at this scale can be attributed to the quantum mechanical nature of particles [1]. Nanomaterials are designed either by breaking down larger particles to nanoscale or built up from the elemental constituents which are respectively known as the top down and the bottom up approaches. Top down approaches consist of the ball-milling process, ion beam lithography, electron beam

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lithography, etc., whereas the bottom up approach consists of the chemical self-assembly, artificial synthesis techniques like those involved in the synthesis of carbon nanotubes [2].

Nanotechnology has influenced various aspects of science and technology as mentioned above. The major applications have been in the field of human health care. It has revolutionized technologies involved in medicine, communication, and genomics that play an important role in improving the present scenario of human health care [3]. There is a continuing endeavor to achieve better, cheaper, and more effective way of medical treatments. It is being widely researched on and many breakthrough technologies have come up in the market.

Nanomedicine, an application of nanotechnology, holds the promise of bringing about future developments in the field of medicine and provides efficient health care. It allows us to understand the functioning of the human body better and fight against a number of serious illnesses like cancer and cardiovascular diseases. Nanomedicine mainly has applications in the diagnosis of a disease and designing efficient and safe drug delivery systems that delivers drug specifically to the target site and helps in the treatment of the disease [1]. For applications in medicine and human health, a wide range of nanomaterials can be devised so that they can interact with the cells and tissues of the human body with a high degree of specificity. The surfaces of these nanomaterials can attach a variety of ligands so that they can be used as imaging agents, drug delivery vehicles, fluorescent tags, and many other important biological tools.

Nanoparticles help in site-specific targeting that increases the efficacy of the drug being administered and reduces the side effects. Due to their small size, they are not easily recognized by the body's immune system and can cross the blood–brain barrier. The different nanoparticles that can be used for drug delivery are liposomes, inorganic nanoparticles, dendrimers, and polymeric nanoparticles. They can also be used for detection, magnetic resonance imaging, and tumor destruction.

Nanomaterials and Their Properties

The specific characteristics and the properties of nanoparticles make them a promising technology for the betterment of human health and care. Nanoparticles have been widely used as a novel therapeutic agent. Many nanoparticles have been used to synthesize different nanoparticle materials.

Gold Nanoparticles

Gold nanoparticles are metal-based nanoparticles that are developed by reduction of gold salts which may either be in the organic phase, aqueous phase, or both. These nanoparticles are associated with a stabilizer that helps in stabilizing the molecule and also provides good ligand-binding capacity. Gold nanoparticles synthesized are categorized into biomolecule-protected, green agent-protected, polymer-protected, and dendrimer-protected gold nanoparticles [4]. The major application is due to their electronic, optical, and thermal properties. Gold nanoparticles ranging between the size 3 and 100 nm are the most preferred as they are stable and their properties can be modified by chemical modification of their surfaces. The most widespread use of these nanoparticles is in diagnostics and detection of biological molecules at low concentration. The interaction with the target molecules induces subtle changes in the emission spectra of the gold nanoparticles which can be detected easily [5]. One such detection technique based on optical properties is the detection of polynucleotides using mercaptoalkyloligonucleotide-modified gold nanoparticle probes. The polymeric network of nanoparticles and oligonucleotides leads to a red to pinkish/purple color change. This technique can detect as low as 10 fM of an oligonucleotide. Some other areas of application of gold nanoparticles are labeling, delivering, and sensing [6].

Magnetic Nanoparticles

Magnetic nanoparticles have wide application in the field of magnetic immunoassays, drug delivery, cell separation, purification, and tissue repair. They are formulated from magnetic materials like Fe_3O_4, Fe_2O_3, and many other ferrites. Nanoparticles can be associated with biorecognition molecules so that they can be used to detect different biomolecules and help in processes like separation and purification. The nature of the surface coatings determine the size and kinetics involved with these particles [7]. Some of the methods used in the development of nanoparticles are co-precipitation, thermal decomposition and reduction, micelles synthesis, etc. One of the prime concerns to be addressed while developing magnetic nanoparticles is to protect them from corrosion. Certain coating strategies are being undertaken to mitigate this problem like polymer coating, silica coating, and carbon coating. It has to be taken under consideration that the coatings being used should be nontoxic and biocompatible, in order to avoid harmful interaction or reaction inside the human body [8].

Magnetic nanosensors have been designed to detect interactions that alter the spin–spin relaxation times of water molecules, which can be detected by NMR/magnetic resonance imaging (MRI) techniques [9]. They can be also be used as fluorescent magnetic nanoparticles for the detection of new tissues in lymphatic vessels of rats. This technique was demonstrated by injecting nanoparticles into the lymph nodes followed by application of magnetic field so that they are taken up by the tissue. The cobalt–ferrite magnetic nanoparticles were coated with silica and a luminescent organic dye was also introduced inside the silica matrix with polyethyleneglycol coat on the outside [10].

Quantum Dots

Quantum dots are semiconductor nanocrystals that are easy to synthesize and have characteristic properties that are between those of bulk semiconductor and discrete molecules. Their diameter ranges from 2 to 10 nm [11]. They have quantized energy levels and the fluorescent properties of these quantum dots are size dependent. As the size of the quantum dot decreases, their band gap increases which results in a greater energy difference between the conduction band and valence band. Due to this significant difference in energy, more energy is required to excite the dot. Consequently, the energy released is higher when the dot returns to its ground state. This results in the emission of light with higher frequency due to which there is a color shift from red to blue in the light emitted that leads to excellent fluorescent properties. Just by changing the size of the dot, a multitude of colors can be emitted. Thus, quantum dots have a broad range of excitation wavelengths [12].

The fluorescent properties of the quantum dots make them suitable for imaging and detection applications. Cancer targeting and imaging has been possible using quantum dots. The semiconductor nanoparticles accumulate at the target sites due to their enhanced permeability and retention at the tumor sites. This has been experimentally proven by carrying out in vivo-targeting studies of human prostate cancer in nude mice [13].

Carbon Nanotubes

Carbon nanotubes are members of the fullerene structural family and are composed of sp^2 bonds which are stronger than sp^3 bonds. They have a cylindrical nanostructure with diameter of the order of few nanometers and have novel electrical, chemical, and mechanical properties which make them useful in the field of medicine. Carbon nanotubes are mainly of two types—single walled carbon nanotubes that consists of a single layer of graphite and multiwalled carbon nanotubes where multiple layers of graphite are rolled within one another to form a tube shape. The transport of electrons in both the nanotubes occur without scattering over long lengths as they have very few defects as a result of which they can carry high currents with negligible heating [14]. Carbon nanotubes have very few defects like dislocations and grain boundaries. The carbon atoms linked by sp^2 bonds do not break when they are bent, they only change their structure. The characteristic features of the carbon nanotubes make them suitable for detection, monitoring, and therapy of diseases. Based on Raman spectroscopy results, it was observed that carbon nanotubes functionalized with polyethylene glycol show blood circulation of 1 day and low uptake by the reitculoendothelial system. These features make them a promising tool for biomedical application [15]. Another example stating the importance of carbon nanotubes is their association with fluorescein and the antifungal drug amphotericin B which show an enhanced biological action of the drug. These modified carbon nanotubes also reduce the toxicity of the drugs administered alone [16].

Liposomes

Liposomes are spherical vesicles where an aqueous core is surrounded by a phospholipid bilayer and cholesterol. The phospholipid consists of a hydrophilic head and two oil-loving tails. The phospholipid that is predominantly used is phosphatidyl choline. Liposomes exhibit several important properties like uniform particle size which is in the range of 50–700 nm and special surface characteristics [17]. They can be classified on the basis of size and the number of layers as small unilamellar, large unilamellar, small multilamellar, and large multilamellar. The size and shape of the vesicles can change with time as their preparations are metastable, i.e., their free enthalpy is not in equilibrium with the environment [18]. The circulating time can be increased by attaching polyethylene glycol molecules to their surface. These molecules protect the liposomes and prevent their clearance. Some of the important application of liposomes can be seen in the field of imaging and drug delivery. Superparamagnetic liposomes have turned out to be good MRI-contrasting agents. In this case, maghemite particles were introduced into a liposomal vesicle synthesized from egg phosphatidylcholine and distearoyl-SN-glycero-3-phosphoethanolamine-*N*-[methoxy(poly(ethylene glycol))-2000]. These liposomes were further functionalized by pegylation. Mouse magnetic resonance angiography followed after 24-h incubation after administration of intravenous injection of the liposomes helped in identifying the efficiency of these contrast agents [19].

Flodots

Flodots are luminescent dye-doped nanoparticles. In flodots, organic or inorganic dyes are introduced in a silica matrix. Tetraethylorthosilicate and phenyltriethoxysilane are utilized to synthesize organic dye-doped flodots. Mainly this kind of characterization is done in order to increase their photostability, increase solubility in aqueous environment, and to reduce dye leakage [20]. The surface of flodots can be further immobilized with biorecognition molecules in order to use them for detection purposes. They have many advantages over the presently used luminescent probes like the quantum dots, etc. and are being widely used in the field of diagnostics, detection, and bioanalysis [21]. The specific use of flodots can be seen in the case of detection of bacteria. In this case, the antibodies against *Escherichia coli* O157:H7 were conjugated with dye-doped silica nanoparticles. Fluorescence resonance energy transfer mediated emission signatures were obtained and different colors were observed under single wavelength excitation (488 nm) from the conjugates [22].

Dendrimers

Dendrimers are large, complex molecules with a well-defined structure. They consist of branches around an inner core. Features like size, shape, branching length, and their surface functionality allow control over the design of these nanoparticles [23]. Polyamidoamine are the mostly used dendrimers for the targeted delivery of drugs and other therapeutic agents. The drug molecules can be loaded either in the inner core or they can be attached covalently to the periphery of the dendrimers. The major application is in the development of MRI contrast agents based on dendrimers [24]. Polyamidoamine dendrimer exhibits properties suitable for using them as MRI-/NIR-contrasting agents and for cancer therapies. Surface-modified dendrimers can be used as nanodrugs against viruses and bacteria. One such dendrimer-derived microbicide (Vivagel) has been developed against HIV and genital herpes. It is based on the multivalency properties of dendrimers. Conjugation of antibodies with polyamidoamine dendrimers can be used as contrast agents, like antibodies against CD14 and prostate-specific membrane antigen. They can be evaluated using flow cytometry and confocal microscopy [25].

Applications in Human Health Care

Human health care is a field that is always looking for improvements in terms of better diagnostics, drug delivery approaches, and imaging technologies and many other aspects of health care. Nanotechnology has enormous scientific and commercial potential in the field of human health care. The greatest significance of using nanotechnology is in the development of effective medical treatments. The technology extends the limits of the present approaches being utilized in the field of human health.

Nanotechnology in Drug Delivery

The designing of an effective drug-targeting system is based on therapeutic efficacy, appropriate concentrations, and a longer circulation time. The drug needs to be released by the targeting system over an extended period of time and should also have target specificity. This specific drug targeting is derived by utilizing the pathophysiological changes in the course of diseases. This is in order to formulate surface ligands which can bind to their respective targets and help in the distribution of the drugs to the respective areas.

Nanocarriers designed to carry drugs like paclitaxel, camptothecin, etc. have helped in increasing their drug efficacy and lowered the toxicity levels. The properties can be attributed to their nano size and leaky vasculatures seen in the case of cancer and inflammatory reactions [26]. In cancers, the vascular permeability increases in order to provide nutrition and oxygen to the growing cells. This phenomenon is known as the enhanced permeability and retention (EPR) effect. The EPR helps in the target-specific delivery of nanomedicines and drugs using nanoparticles because the permeability for lipids and such nanoparticles increases. Tumor-specific drugs can be loaded onto lipid-based nanoparticles for better target-specific delivery of drugs [27]. The systems available in the body itself can be exploited for better deliveries of drug–nanoparticle combinations.

Drug conjugates formed with the help of ampiphilic polymers, water-insoluble polymers, liposome, carbon nanotubes, etc. have the ability to cross the blood–brain barriers and effectively increase the drug absorption. They can cross other biological barriers like that of cornea and the skin [28]. Blood–brain barrier, a neuroprotective shield surrounds the brain in all the directions to resist the entry of high molecular weight molecules. This job is also done by the cerebrospinal fluid which ultimately may reduce the efficacy of various drug molecules. Nanoparticles are efficient drug delivery system as they can pass through these relatively impermeable barriers. The target organ being brain, several nanoparticles can also potentially increase the transport of the drugs like insulin, growth factors, albumin, etc. Functionalization of nanoparticles also needs to be done so that the nanoparticles can retain the drug until they reach the target organ. This process is done by using various hydrophilic surfactants like Tween 80 [29].

There are two kinds of drug-targeting methods—passive targeting and active targeting. Passive targeting utilizes the EPR to target the drug molecules, whereas in active targeting, the nanopartcles are directed towards certain molecules by providing targeting moieties to them. The nanoparticles in the case of active targeting are directed towards carbohydrates, receptors, or specific antigens. Some of the materials that are being used for formulating these nanoparticles are lectin, polyacrylamide, polyvinyl alcohol, etc. [30]. Drug delivery for ocular diseases is problematic as there is low bioavaibility due to various anatomical and pathophysiological barriers but nanotechnology is one such field that is been looked into for solutions. Various nanoparticle systems are being applied like nanoparticles, dendrimers, niosomes, and liposomes to address this issue [31].

Bioavaibility of oral drugs is also low due to the low pH of the stomach and poor absorption by the intestinal mucosa [32]. Nanotechnology-based drug delivery systems have been designed to overcome these barriers like that of gliadin nanoparticles that show significant increase in bioavaibility and bioadhesion in the intestinal mucosa [33]. Poly (methylvinylether-co-maleic anhydride) nanoparticles, nanoparticles coated with albumin, and nanoparticles treated with albumin and 1,3-diaminopropane show an increase in bioadhesion [34]. Polyamidoamines (PAMAMs) are dendrimers that are used as vectors in drug delivery. They have many biomedical applications due to their unique properties [35]. Many supercritical fluid technologies also have wide applications in the development of nanotechnology-based drug formulations [36]. Micro and nano electromechanical systems-based drug delivery systems can help in controlled, long, and sustained release of drugs. It is a commercially feasible technology [37]. Colloidal gold nanoparticles are being used and further investigated for delivery of drugs to tumor cells. The drugs that have been delivered successfully using this technology are paclitaxel and tumor necrosis factor [38].

Applications of nanotechnology are promising in mitigating many problems of the present drug delivery systems and further advancement will bring about more breakthrough technologies. The extensive study of the disease will help to outline the features on the basis of which the drugs can be targeted using the nanovehicles.

Nanotechnology in Imaging

Molecular imaging is an emerging technology which is intended to improve the accuracy of disease diagnosis. This is a promising technology as it helps in the detection of the disease and it also helps in deciding whether the therapy provided for the treatment is beneficial and complication free. The growth of nanotechnology has increased the opportunities of molecular imaging in the characterization of disease pathology. Nanotechnology has introduced a number of nanoparticles that are used in targeted diagnostic imaging and have expanded the potential of imaging. These nanoparticles are very sensitive and specific to detect the sparse biomarkers associated with any disease and provide for an early detection of the disease. The nanoparticles used in molecular imaging are liposomes, dendrimers, gold, iron oxide, and perfluorocarbon nanoparticles [39, 40].

The nanoparticles are used as contrast agents in MRI. Nanoparticles when used as MRI contrast agents work by shortening the spin–lattice relaxation time T1 and spin–spin relaxation time T2 resulting in the formation of a sharper and brighter image [41]. These nanoparticles have superparamagnetic properties and they can change the spin–spin relaxation time of the neighboring water molecules due to which they can monitor the expression of genes and detect tumors, atherosclerotic plaques, tissue inflammation, arthritis, etc. [9, 42]. They can be actively targeted or passively targeted to detect the epitopes of our interest and to differentiate between the normal and the diseased tissue. Passive targeting is non-targeted directing where the contrast agents are easily recognized as foreign which leads to the rapid clearance of the agents by the reticuloendothelial system. Active targeting is ligand-directed, site-specific targeting of the contrast agents where the ligands are able to identify a specific pathological tissue. Antibodies, peptides, polysaccharides, aptamers, and drugs are wide variety of ligands that may be utilized. Ligands can be attached either covalently or non-covalently and then further polyethylene glycol can be integrated to alter the surface of the nanoparticles. This modification can delay the removal of these agents from the body and allow the ligands to go and bind to the target tissue [43, 44].

Nanoparticles have desired properties like small size, high surface area-to-volume ratio, long circulating hours, high affinity for the target tissue, easy production, less toxicity, and immunogenicity which make them ideal contrast agents [45]. The small size of these particles makes them less efficient in activating the complement system due to which they are cleared slowly from the blood leading to their long circulating half-life. Nanoparticles also have multiple binding sites due to which they enhance avidity for the target tissue. This leads to specific binding and permits longer binding of these agents to the target site that allows for imaging at appropriate times.

Nanotechnology has also brought about the possibility of intracellular imaging by using nanoparticles like quantum dots where the high-fluorescence intensity of these quantum dots allows for tracking the cells throughout the body. They are more appealing than the conventional fluorescent labels because they are more stable which allow for images that are sharper and crisper over long periods of time and because of their ability to detect multiple signals at the same time. They are also brighter due to which a small number of quantum dots are sufficient in producing the desired signal. Quantum dots are also used as fluorescent tags and have shown great promise in the detection and diagnosis of various diseases. Their small size allows them to easily enter the cells and interact with the biomolecules present within the cell [46]. This is possible due to their larger surface area and their broad range of excitation wavelengths. Quantum dots have proteins attached on their surface so that they can identify the distinct proteins that are present on the diseased tissues [47].

Atomic force microscopy is one important tool for imaging matter at the nanoscale and provides a high-resolution three-dimensional image. This technique may also require the contrast agents that are employed in MRI. In atomic force microscopy, a microscale cantilever with a sharp tip is used as a probe that scans the specimen surface. The attractive and repulsive forces between the probe and the surface that is at a short distance of 1–10 nm cause a deflection of the cantilever. This deflection can be quantified by the beam bounce method where a laser beam on top of the cantilever into a range of photodiodes and provide a three-dimensional profile of the scanned surface. Developments in atomic force microscopy have allowed for tumor detection, detection of erythrocytes influenced by diabetes, and to study the structure of a C-reactive protein that is a risk for coronary artery disease and peripheral arterial disease [48]. The applications of nanotechnology in molecular imaging are creating the way for personalized medicine and will undoubtedly bring about a huge revolution and a huge transformation in the diagnosis, treatment, and prevention of a disease in the future and will provide ways that will improve a patient's health and will benefit the patients even more.

Nanotechnology in Gene Delivery

Gene therapy is a technique involving alteration, removal, or insertion of a gene at particular loci in order to treat various genetic disorders. Vectors are required to transfer the gene to their desired location. The vectors can be viral or nonviral in origin. The viral vectors include adenoviruses, retroviruses, lentiviruses, and adeno-associated viruses [49]. The viral vectors are quite useful in utilizing the natural mechanism of infection [50].

Gene delivery is the method of replacing a defective gene with a normal one or delivering genes into the diseased cells for curing and treating the diseases. Earlier it was a method for the treatment of hereditary diseases but now it has a very important role in the treatment of diseases like cancer [51]. There are certain limitations of gene therapy that can be overcome by nanotechnology. Nanotechnology has introduced nonviral vectors like liposomes and dendrimers that are less immunogenic than the currently used viral vectors [52]. The nonviral vectors are generally cationic in nature and encapsulate the negatively charged DNA by electrostatic interactions. Nanoparticles make good nonviral vector systems because they are safe, simple to use, and easy to produce. Their transfection efficiency is less than the viral vectors but certain adjustments can be made like the attachment of ligands that can make them promising gene delivery systems [53, 54].

Dendrimers are efficient gene delivery systems. They have the ability to protect DNA from the action of DNAse enzyme. The most commonly used dendrimer for gene delivery is PAMAM because of its high transfection efficiency. The transfection efficiency can be further enhanced by heat treatment in solvents like water and butanol. The heat treatment increases the flexibility which enables the dendrimers to become compact when compounded with DNA [55]. Polyamidoamine contains a primary amino group on their surface that binds the DNA and promotes their uptake by the cells and tertiary amino groups that are present within permit the release of DNA in the cytoplasm.

Liposomes have also been explored as delivery systems for successful gene transfer and have certain advantages as gene delivery carriers. Their size can be easily controlled and modified to add a targeting agent. An obstacle for using liposomes as gene delivery systems is their low efficiency of encapsulating DNA. This low efficiency can be overcome by using cationic liposomes. They consist of lipid bilayers that are positively charged that combine spontaneously with the negatively charged DNA by electrostatic and hydrophobic interactions. In order to improve transfection efficiency, these liposomes are mixed with cholesterol and further modified with functional ligands [56].

Conclusion

Nanotechnology is a vast field which has its applications in human health care, medicine, electronics, information and communication, etc. The developments in nanotechnology have brought about many benefits associated with health care and medicine. They have introduced new diagnostic and therapeutic techniques that have brought about a revolution in early disease detection and the treatment of various diseases. These innovative strategies has helped in overcoming the limitations associated with the conventional strategies involved in treating the diseases and has brought about a breakthrough which is undoubtedly going to be further developed in the future.

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